

CLAIMS

1. A method for the culture of mammalian cells comprising the steps of:
 - i) providing a cell culture vessel comprising:
 - 5 a) mammalian cells;
 - b) a cell culture support comprising a substrate wherein said substrate comprises a cell culture surface wherein said surface comprises a polymer of an acid monomer and attached thereto, fibroblast feeder cells
 - 10 c) cell culture medium sufficient to support the growth of said mammalian cells wherein said medium does not include serum; and
 - iii) providing cell culture medium and conditions which promote the proliferation of said mammalian cells.
- 15 2. A method according to Claim 1 wherein said mammalian cells are human.
3. A method according to Claim 1 or 2 wherein said mammalian cells are maintained in culture in an un-differentiated state.
- 20 4. A method according to any of Claims 1-3 wherein said mammalian cells are selected from the group consisting of: epidermal keratinocytes; dermal fibroblasts; adult skin stem cells; embryonic stem cells; melanocytes, corneal fibroblasts, corneal epithelial cells, corneal stem cells; intestinal mucosa fibroblasts, intestinal mucosa keratinocytes, oral mucosa fibroblasts, oral mucosa keratinocytes, urethral fibroblasts
25 and epithelial cells, bladder fibroblasts and epithelial cells, neuronal glial cells and neural cells, hepatocyte stellate cells and epithelial cells.
5. A method according to Claim 4 wherein said mammalian cells are autologous
30 keratinocytes.

6. A method according to any of Claims 1-5 wherein the number of said mammalian cells and said fibroblast cells is at a ratio of about between 1:1-5:1.
7. A method according to Claim 6 wherein said ratio is about 5:1.
- 5 8. A method according to any of Claims 1-7 wherein said mammalian cells are seeded at about 0.75×10^4 cells/mm².
9. A method according to any of Claims 6 – 8 wherein said mammalian cells are
10 keratinocytes.
10. A method according to any of Claims 1-9 wherein said substrate comprises a non-porous polymer.
- 15 11. A method according to any of Claims 1-9 wherein said substrate is a solid phase substrate.
12. A method according to any of Claims 1-9 wherein said substrate is a porous material.
- 20 13. A method according to Claim 12 wherein said material is a woven material.
14. A method according to Claim 12 wherein said material is a non-woven material.
- 25 15. A method according to any of Claims 1-14 wherein said cell culture surface comprises a polymer comprising an acid content of at least 2%.
16. A method according to any of Claims 1-15 wherein said surface comprises a
30 polymer comprising an acid content between about 2-20%.

17. A method according to any of Claims 1-14 wherein said surface comprises a polymer comprising an acid content greater than 20% .
18. A method according to Claims 15 or 16 wherein said polymer comprises
5 an acrylic acid monomer with at least 2% acid content.
19. A method according to Claim 18 wherein said acid content is between 2% and 10%.
- 10 20. A method according to Claim 19 wherein said acid content is about 4-5%.
21. A method according to any of Claims 1-20 wherein said polymer comprises an acid co-polymer.
- 15 22. A method according to any of Claims 1-21 wherein said fibroblast feeder cells are non-proliferative.
23. A method according to Claim 22 wherein said fibroblast feeder cells are rendered non-proliferative by lowering the calcium concentration of the growth
20 medium.
24. A method according to any of Claims 1-23 wherein said feeder cells are human fibroblasts.
- 25 25. A method according to Claim 24 wherein said fibroblasts are dermal or oral fibroblasts.
26. A method according to Claim 24 or 25 wherein said fibroblasts are autologous.
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27. A cell culture vessel comprising: a cell culture support comprising a substrate wherein said substrate comprises a cell culture surface wherein said surface comprises a polymer of an acid monomer and attached thereto, fibroblast feeder cells.

5 28. A vessel according to Claim 27 wherein said vessel further comprises mammalian cells and cell culture medium which medium does not include serum.

29. A vessel according to Claim 28 wherein said mammalian cells are selected from the group consisting of: epidermal keratinocytes; dermal fibroblasts; adult skin
10 stem cells; embryonic stem cells; melanocytes, corneal fibroblasts, corneal epithelial cells, corneal stem cells; intestinal mucosa fibroblasts, intestinal mucosa keratinocytes, oral mucosa fibroblasts, oral mucosa keratinocytes, urethral fibroblasts and epithelial cells, bladder fibroblasts and epithelial cells, neuronal glial cells and neural cells, hepatocyte stellate cells and epithelial cells.

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30. A vessel according to Claim 29 wherein said mammalian cells are keratinocytes, preferably autologous keratinocytes.

31. A method to treat a cell culture vessel comprising the steps of:

- 20 i) providing at least one acid monomer source in a gas feed;
ii) creating a plasma of said acid monomer; and
iii) bringing into contact a cell culture vessel with said plasma monomer to provide a cell culture vessel comprising an acid polymer.

25 32. A method according to Claim 31 wherein said acid monomer source comprises 30-99% acid monomer.

33. A method according to Claim 31 wherein said acid monomer source consists of a 100% acid monomer source.

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34. A method according to Claim 33 wherein said acid monomer source consists of a 100% acrylic acid.

35. A method to treat a cell culture vessel comprising the steps of:

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- (i) providing a selected ratio of an acid containing monomer and a hydrocarbon in a gas feed;
 - (ii) creating a plasma of said mixture;
 - (iii) bringing into contact a cell culture vessel with said plasma mixture to provide a cell culture surface comprising an acid co-polymer.

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36. A method according to Claim 35 wherein said plasma is created by means of electrical power input coupled by means of a copper coil or bands.

37. A method to culture mammalian cells on a therapeutic vehicle comprising the steps of:

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- i) providing a preparation comprising;
 - a) mammalian cells;
 - b) a therapeutic vehicle wherein said vehicle comprises a substrate which comprises a surface wherein said surface comprises a polymer of an acid monomer and attached thereto, fibroblast feeder cells;
 - c) cell culture medium sufficient to support the growth of said mammalian cells wherein said medium does not include serum; and
- ii) providing cell culture conditions which promote the proliferation of said mammalian cells on said therapeutic vehicle.

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38. A method according to Claim 37 wherein said mammalian cells are human.

39. A method according to Claim 37 or 38 wherein said mammalian cells are selected from the group consisting of: epidermal keratinocytes; dermal fibroblasts; adult skin stem cells; embryonic stem cells; melanocytes, corneal fibroblasts, corneal epithelial cells, corneal stem cells; intestinal mucosa fibroblasts, intestinal mucosa

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keratinocytes, oral mucosa fibroblasts, oral mucosa keratinocytes, urethral fibroblasts and epithelial cells, bladder fibroblasts and epithelial cells, neuronal glial cells and neural cells, hepatocyte stellate cells and epithelial cells.

5 40. A method according to any of Claims 37-39 wherein said mammalian cells are autologous.

41. A method according to Claim 39 or 40 wherein said mammalian cells are keratinocytes.

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42. A method according to any of Claims 37-41 wherein said fibroblast feeder cells are human.

43. A method according to any of Claims 42 wherein said fibroblast feeder cells
15 are dermal fibroblasts or human oral fibroblasts.

44. A method according to Claim 42 or 43 wherein said feeder cells are autologous.

20 45. A method according to any of Claims 37-44 wherein the number of said mammalian cells and said fibroblast cells is at a ratio of about between 1:1 – 5:1.

46. A method according to Claim 45 wherein said ratio is about 5:1.

25 47. A method according to Claim 45 or 46 wherein said mammalian cells are keratinocytes and are in a ratio of about 5:1 with said fibroblast cells.

48. A method according to any of Claims 37-47 wherein said mammalian cells are seeded at about 0.75×10^4 cells/mm²

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49. A method according to any of Claims 45-48 wherein said therapeutic vehicle comprises a substrate composed of a polymeric material wherein the ratio of mammalian cells to fibroblast cells is about 5:1.

5 50. A method according to Claim 49 wherein said substrate is composed of a vinyl polymer.

51. A method according to Claim 50 wherein said vinyl polymer is selected from the group consisting of: polyvinyl chloride, polyvinyl acetate, polyvinyl alcohol.

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52. A therapeutic vehicle obtainable by the method according to any of Claims 37-51.

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